



## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Hilaly *et al.*

Appl. No. 10/824,358

Filed: April 15, 2004

For: **Process for Producing High Purity  
Isoflavones**

Confirmation No. 6313

Art Unit: 1761

Examiner: Anthony J. Weier

Atty. Docket: 1533.6040002

**Declaration of Ahmad K. Hilaly, Bob Sandage and John Soper  
Under 37 C.F.R. § 1.131**Commissioner for Patents  
Washington, D.C. 20231

Sir:

The undersigned, Ahmad K. Hilaly, Bob Sandage and John Soper, declare and state that,

1. We are the inventors of the above-captioned application, U.S. Appl. No. 10/824,358, filed April 15, 2004 (the "'358 application"). We are also the inventors of U.S. Appl. No. 10/409,683, filed April 9, 2003 (the "'683 application"), and U.S. Provisional Patent Appl. No. 60/271,129 (the "'129 application"), filed on April 10, 2002.
2. The '358 application is a divisional application of the '683 application, which claims the benefit of the '129 application.
3. Prior to January 9, 2002, we, the inventors, had conceived of our invention in the United States, as claimed in the subject application, and diligently proceeded to file a patent application as evidenced by the following:
4. Exhibit A is twelve (12) pages of a dated document (date redacted) showing data from isoflavone purification experiments that generally correspond to the pending application, and which represent examples of the claimed invention. Exhibit A was prepared by us prior to January 9, 2002. This is earlier than the publication date of Izumi *et al.*, Japanese Patent Application No. P2002-184802A (January 9, 2002) and the publication date of Katayama *et al.*, Japanese Patent Application No. P2002-80474A (March 19, 2002).

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5. We began the protocol that results in the high purity isoflavone enriched fraction of claimed invention prior to January 9, 2002. High purity isoflavone enriched fractions were prepared between August and November, 2001. An invention disclosure form was submitted to our legal department and the above-mentioned provisional application was filed April 10, 2002.

6. As the persons signing below, we hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issue thereupon.

11-17-04

Date

Ahmad Hilaly  
Ahmad K. Hilaly11-18-04

Date

Robert Sandage  
Bob Sandage11/17/04

Date

John Soper  
John Soper

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# ISOFLAVONE pH VS. ADSORPTION TESTS

THE PURPOSE OF THESE TESTS IS TO ASSESS THE PERFORMANCE OF OTHER ANIONIC RESINS  
IN OUR ISOFLAVONE PROCESS. THESE WILL BE OUR STANDARD S&D TESTS

## EXPERIMENTAL DATA

EXPERIMENT NUMBER: 67A  
FEED: pH ADJUSTED @ PLANT // 100K MWCO // PLANT W/F PERM (GMO FEED)  
COND'S: pH 8.95  
12 MLS/MIN  
60 DEG C  
COLUMN TEMP: 60 DEG C  
RESIN: MITSUBISHI SAP 12A  
DATE: 6/8/01  
LOADING: 1176.5 g/l resin BED VOLUME: 100 mls  
ISOFLAVONE CONC. IN FEED: 1176.47 PPM  
ISOFLAVONE CONC. IN FEED: 1176 g/l  
BED VOLUMES FED: 10 B.V.

	Volume (L)	ISOFLAVONE (mg/kg)	D/S (g/kg)	Vol/d	Purity %	Yield %
FEED	1.0	1176.5	1176.5	0.2	0.2	0.2
RAPPINATE	1.0	331.2	331.2	0.2	28.15	38.17
RINSE NO. 1	0.3	393.0	393.0	1.0	10.02	
PRODUCT	1.0	2195.9	3.0	72.4	186.65	
PRODUCT 2X		1194.4	1.7	71.4	0.00	
RINSE NO. 2	0.3	32.4	0.4	8.1	0.85	
2.5% CAUSTIC	0.25	135.6	22.2	0.6	2.66	
RINSE NO. 3	0.5	9.1	0.1	0.1	0.25	

EXPERIMENT NUMBER: 67B  
FEED: pH ADJUSTED @ PLANT // 100K MWCO // PLANT W/F PERM (GMO FEED)  
COND'S: pH: 8.95  
12-MLS/MIN  
60 DEG C  
RESIN: MITSUBISHI SAP 12A  
LOADING: 1176.5 g/l resin BED VOLUME: 100 mls  
ISOFLAVONE CONC. IN FEED: 1176.47 PPM  
ISOFLAVONE CONC. IN FEED: 1176 g/l  
BED VOLUMES FED: 10 B.V.

	Volume (L)	ISOFLAVONE (mg/kg)	D/S (g/kg)	Vol/d	Purity %	Yield %
FEED	1.0	1176.5	1176.5	0.2	0.2	0.2
RAPPINATE	1.0	331.2	331.2	0.2	28.15	38.17
RINSE NO. 1	0.3	393.0	393.0	0.9	0.67	
PRODUCT	0.5	711.05	1.4	50.1	30.22	
PRODUCT 2X		353.45	0.7	53.4	0.00	
RINSE NO. 2	0.3	137.24	1.3	10.7	3.50	
2.5% CAUSTIC	0.25	41.81	22.8	0.2	0.89	
RINSE NO. 3	0.3	6.0	0.0	0.1	0.15	

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Date

Recorded/Invented by

Date

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## ISOFLAVONE pH VS. ADSORPTION TESTS

THE PURPOSE OF THESE EXPERIMENTS IS TO A) FOLLOW THE PILOT C-STEP TO ASCERTAIN SYSTEM PERFORMANCE VS. SINGLE COLUMN AND B.) TO FURTHER TEST THE SAF 12A RESIN FROM MITSUBISHI FOR USE IN THIS PROCESS.

## EXPERIMENTAL DATA

EXPERIMENT NUMBER: 68A  
 FEED: pH ADJUSTED @ PLANT // 100K MWCO // PLANT U/F PERM. (GMO FEED)  
 CONDITIONS: FEED RATE: 12 MLS/MIN  
 COLUMN TEMP.: 60 DEG C  
 RESIN: A-2X-MP (PLANT RESIN)  
 ISOFLAVONE CONC. IN FEED: 1129.59 PPM  
 ISOFLAVONE CONC. IN FEED: 1130 g/L  
 BED VOLUMES FED: 10 B.V.

	Volume (L)	Isoflavone (mg/kg)	D/S (g/kg)	Vol/g	Purity %	Yield %
FEED	1.0	1129.6	100.2		1.1	100.00
RINSE NO. 1	0.3	379.6	34.2		1.1	10.05
PRODUCT	1.0	1683.5	2.5		67.8	149.03
PRODUCT 2X		876.0	1.1		55.0	0.00
RINSE NO. 2	0.3	28.9	0.1		28.9	0.77
2.5% CAUSTIC	0.25	136.8	121.6		0.1	3.03
RINSE NO. 3	0.3	21.5	77.2		0.0	0.57
TOTAL						166.45

EXPERIMENT NUMBER: 68B  
 FEED: pH ADJUSTED @ PLANT // 100K MWCO // PLANT U/F PERM. (GMO FEED)  
 CONDITIONS: FEED RATE: 12 MLS/MIN  
 COLUMN TEMP.: 60 DEG C  
 RESIN: MITSUBISHI SAF 12A

ISOFLAVONE CONC. IN FEED: 1129.59 PPM  
 ISOFLAVONE CONC. IN FEED: 1130 g/L  
 BED VOLUMES FED: 10 B.V.

	Volume (L)	Isoflavone (mg/kg)	D/S (g/kg)	Vol/g	Purity %	Yield %
FEED	1.0	1129.6	100.2		1.1	100.00
RINSE NO. 1	0.3	366.9	29.7		0.3	3.72
PRODUCT	0.5	551.2	1.2		45.6	24.40
PRODUCT 2X		261.3	0.3		74.7	0.00
RINSE NO. 2	0.3	163.7	1.1		15.5	4.35
2.5% CAUSTIC	0.25	63.8	97.1		0.1	1.41
RINSE NO. 3	0.3	11.9	45.6		0.0	0.32
TOTAL						52.28

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Witnessed and Understood by Me

Date

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## ISOFLAVONE pH VS. ADSORPTION TESTS

THE PURPOSE OF THESE EXPERIMENTS IS TO: A.) DETERMINE THE EFFECT OF RUNNING STRIPPER PRODUCT THROUGH AS A RINSE 1A, AND B.) DETERMINE THE FEASIBILITY OF RUNNING OUR STRIPPER PRODUCT THROUGH THE RESIN AGAIN, TO INCREASE PURITY. STRIPPER PRODUCT IS C-SEP PRODUCT (FROM THE PLANT) THAT HAS HAD THE ETHANOL REMOVED.

## EXPERIMENTAL DATA

EXPERIMENT NUMBER: 70A  
 FEED: PH ADJUSTED @ PLANT / 100% MDC / PLANT 317 TERM: (GMO FEED)  
 CONDITIONS: pH 6.7  
 FEED RATE: 12 MLS/MIN  
 COLUMN TEMP: 60 DEG C  
 RESIN: A-2X-MP (PLANT RESIN)

DATE: 11/13/04  
 LO. NO.: 1000  
 ISOFLAVONE CONC. IN FEED: 969.1 mg/kg  
 ISOFLAVONE CONC. IN FEED: 1.03 g/L  
 BED VOLUME: 10 B.V.  
 STRIPPER PROD: 969.1 mg/kg Isoflavone

FEED	Volume (L)	Isoflavone (mg/kg)	D/S (g/kg)	Vol/g	Purity %	Yield %
FEED	1.0	1090.9	89.1		1.2	100.00
RAFFINATE	1.0	275.9	80.7		0.3	22.77
STRIP. PRODUCT	0.3	47.2	22.3		2.0	0.00
DI RINSE 1B	0.3	283.1	1.2		21.0	64.00
PRODUCT	1.0	1722.5	5.4		60.8	140.66
PRODUCT 2X		864.9	1.7		49.5	0.00
RINSE NO. 2	0.3	46.2	0.4		12.8	1.13
2.5% CAUSTIC	0.25	241.6	23.6		1.0	4.03
RINSE NO. 3	0.3	22.1	9.1		0.2	0.54

EXPERIMENT NUMBER: 70B  
 FEED: STRIPPER PRODUCT  
 CONDITIONS: pH ADJUSTED TO 9.55; 2L OF STRIP. PROD. DILUTED WITH 2L OF DI H2O  
 FEED RATE: 12 MLS/MIN  
 COLUMN TEMP: 60 DEG C  
 RESIN: A-2X-MP (PLANT)

LOADING: 969.1 mg/kg  
 ISOFLAVONE CONC. IN FEED: 1.03 g/L  
 ISOFLAVONE CONC. IN FEED: 0.969 g/L  
 BED VOLUMES FED: 10 B.V.

FEED	Volume (L)	Isoflavone (mg/kg)	D/S (g/kg)	Vol/g	Purity %	Yield %
FEED	1.0	969.1	2.3		41.7	100.00
RAFFINATE	1.0	11.4	0.6		1.18	
RINSE NO. 1	0.3	47.0	0.3		3.6	0.45
PRODUCT	0.5	263.3	1.5		86.8	25.2
PRODUCT 2X		582.3	0.8		75.9	0.00
RINSE NO. 2	0.3	82.1	0.2		32.8	2.34
2.5% CAUSTIC	0.25	47.0	23.2		0.2	1.21
RINSE NO. 3	0.3	6.5	9.4		0.1	0.21

TOTAL 71.80

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## ISOFLAVONE HIGH PURITY STEP WISE ELUTION TESTS

THE PURPOSE OF THESE EXPERIMENTS IS TO

TERMINE RESINS, (ADS 600 & PCA-11) GIVE A HIGHER PURITY PRODUCT FROM PLANT FINAL PRODUCT UTILIZING A GRADIENT  
ELUTION OF 35% ETHOH FOLLOWED BY A 70% ELUTION WASHING

## EXPERIMENTAL DATA

EXPERIMENT NUMBER

DATE: 10/16/04

FEED: PLANT FINAL PRODUCT (DILUTED 4gm WITH 200 ml H<sub>2</sub>O PH to 9.3)  
HEATED TO 70 C

CONDITIONS

FEED RATE

COND'S PH "ADJUSTED"

9.7

COLUMN TEMP

12 ML6/MIN

RESIN:

ADS 600

65 DEG C

D/S

DATE:

10/16/04

LOADING:

7 g/L resin

BED VOLUME

100 ml

ISOFLAVONE CONC. IN FEED: 3615 PPM

ISOFLAVONE CONC. IN FEED: 0.362 g/L

BED VOLUMES FED: 18 B.V.

	Volume (L)	isoFlavone (mg/kg)	D/S (g/kg)	Vol/g	Purity %	Yield %	D/S Account.
FEED	1.5	3615	2.4		15.1	100.00	100.00
KAFFINATE	1.5	22.9	4.9		0.6	6.33	200.00
RINSE NO. 1	0.3	26.3	0.5		5.3	1.21	3.47
COMPOSITE (1st 3 fractions) 35%	0.3	3653.0	6.9		52.9	188.42	47.92
PRODUCT 2X	0.3	1509.0	0.9		15.5	0.00	0.00
COMPOSITE (4th 5 fractions) 35%	0.3	2725.0	15.4		50.5	203.39	62.50
PRODUCT 2X	0.3	1343.6	9.0		14.9	0.00	0.00
COMPOSITE 70%	0.5	12.6	0.8		1.8	0.97	9.26
PRODUCT 2X	0.3	6.9	1.4		0.5	0.00	0.00
RINSE NO. 2	0.3	0.8	12		0.1	0.04	8.33
2.5% CAUSTIC	0.3	1.5	84.2		0.0	0.06	480.56
RINSE NO. 3	0.3	2.0	108.5		0.0	0.08	759.58

## FREEZE DRIED RESULTS (FROM 1ST 3 FRACTIONS)

Total Isoflav. ppm: 31025  
Daidzin, ppm: 173256  
Genistin, ppm: 31025

TOTAL

203.34 1606.02

EXPERIMENT NUMBER

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FEED: PLANT FINAL PRODUCT (DILUTED 4gm WITH 2L DI H<sub>2</sub>O PH to 9.3)  
HEATED TO 70 C

CONDITIONS

FEED RATE

COND'S PH "ADJUSTED"

9.3

COLUMN TEMP

12 ML6/MIN

RESIN:

PCA-11

65 DEG C

LOADING: 6507 g/L resin

BED VOLUME:

100 ml

ISOFLAVONE CONC. IN FEED: 36150 PPM

ISOFLAVONE CONC. IN FEED: 0.362 g/L

BED VOLUMES FED: 18 B.V.

	Volume (L)	isoFlavone (mg/kg)	D/S (g/kg)	Vol/g	Purity %	Yield %	D/S Account.
FEED	1.0	3615	2.4		15.1	100.00	100.00
KAFFINATE	1.5	22.9	4.9		0.6	1.63	1.63
RINSE NO. 1	0.3	26.3	0.5		12	0.22	1.25
COMPOSITE (1st 3 fractions) 35%	0.3	1778	3.4		82.1	81.74	25.61
PRODUCT 2X	0.3	815.4	1.0		81.5	0.00	0.00
COMPOSITE (4th 5 fractions) 35%	0.5	1874.1	3.0		82.5	164.01	34.72
PRODUCT 2X	0.3	910.9	12		75.9	0.00	0.00
PRODUCT (70%)	0.5	721.6	1.0		72.2	55.45	11.57
PRODUCT 2X	0.3	375.6	0.4		93.9	0.00	0.00
RINSE NO. 2	0.3	18.8	0.0		0.0	0.77	0.00
2.5% CAUSTIC	0.3	1.5	84.2		0.0	0.74	879.77
RINSE NO. 3	0.3	2.0	108.5		0.0	0.74	948.25

## FREEZE DRIED RESULTS (FROM 1ST 3 FRACTIONS)

Total Isoflav. ppm: 391605  
Daidzin, ppm: 199606  
Genistin, ppm: 134824  
Ratio: 0.68

TOTAL

203.34 1606.02

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## ISOFLAVONE HIGH PURITY STEP-WISE ELUTION TESTS

THE PURPOSE OF THESE EXPERIMENTS IS TO  
DETERMINE IF RESINS (ADD 600.50 g/L 5% CAUSTIC) GIVE A HIGHER PURITY PRODUCT FROM A DISSOLVED FINAL UK PRODUCT  
UTILIZING A 50% INITIAL ELUTION OF 5% CAUSTIC FOLLOWED BY A 70% ELUTION WASHING

## EXPERIMENTAL DATA

EXPERIMENT NUMBER 5  
DATE 10/22/04  
FEED PLANT FINAL PRODUCT (DILUTED 4x WITH XL DI H2O PH 12.92)  
CONDENS. 12.000000  
COLUMN TEMP. 60.000000  
RESIN ADD 600  
LOADING 13.00 g/L resin BED VOLUME 100 mL  
ISOFLAVONE CONC IN FEED 692.5 PPM  
ISOFLAVONE CONC IN FEED 0.69 g/L  
BED VOLUMES FED 13.0 V

FEED	VOLUME (L)	ISOFLAVONE (mg/L)	DYE (g/L)	Vol/Lg	Purity %	Yield %	DYE ACCOUNT
19	1.0	692.5	1.0	24.5	100.0	70.0	
WASH RATE	1.0	17.6	1.0	1.2	12.6		
RINSE NO. 1	0.5	27.5	0.5	0.0	0.0		
PRODUCT (10%)	0.5	1000.3	2.3	0.0	0.0		
PRODUCT (10%)	0.5	62.2	0.1	0.0	2.4	14	
PRODUCT 2X	0.5	32.0	0.1	0.0	0.0	0.0	
RINSE NO. 2	0.5	2.6	0.0	0.0	0.1	0.0	
2.5% CAUSTIC	0.5	1.0	46.7	0.0	0.0	197.0	
RINSE NO. 3	0.5	2.2	67.6	0.0	0.1	561.6	
TOTAL					100.0	877.5	

EXPERIMENT NUMBER 6  
DATE 10/22/04  
FEED PLANT FINAL PRODUCT (DILUTED 4x WITH XL DI H2O PH 12.92)  
CONDENS. 12.000000  
COLUMN TEMP. 60.000000  
RESIN ADD 600  
LOADING 13.00 g/L resin BED VOLUME 100 mL  
ISOFLAVONE CONC IN FEED 692.5 PPM  
ISOFLAVONE CONC IN FEED 0.69 g/L  
BED VOLUMES FED 20.0 V

FEED	VOLUME (L)	ISOFLAVONE (mg/L)	DYE (g/L)	Vol/Lg	Purity %	Yield %	DYE ACCOUNT
19	1.0	692.5	1.0	24.5	100.0	100.0	
WASH RATE	1.0	17.6	1.0	1.2	12.6		
RINSE NO. 1	0.5	27.5	0.5	0.0	0.0		
PRODUCT 2X	0.5	1001.2	2.0	0.0	0.0	20.0	
PRODUCT (70%)	0.5	733.5	1.2	6.1	0.0	0.0	
PRODUCT 2X	0.5	1197.7	1.2	69.0	45.1	10.0	
PRODUCT 2X	0.5	603.1	0.7	60.2	0.0	0.0	
RINSE NO. 2	0.5	50.9	0.2	29.5	1.5	17	
2.5% CAUSTIC	0.5	21.6	52.0	0.0	0.5	229.4	
RINSE NO. 3	0.5	15.2	53.1	0.0	0.5	499.1	
TOTAL					104.8	100.0	

EXPERIMENT NUMBER 7  
DATE 10/22/04  
FEED PLANT FINAL PRODUCT (DILUTED 4x WITH XL DI H2O PH 12.92)  
CONDENS. 12.000000  
COLUMN TEMP. 65.000000  
RESIN ADD 600  
LOADING 13.00 g/L resin BED VOLUME 100 mL  
ISOFLAVONE CONC IN FEED 692.5 PPM  
ISOFLAVONE CONC IN FEED 0.69 g/L  
BED VOLUMES FED 13.0 V

FEED	VOLUME (L)	ISOFLAVONE (mg/L)	DYE (g/L)	Vol/Lg	Purity %	Yield %	DYE ACCOUNT
19	1.0	692.5	1.0	24.5	100.0	75.7	
WASH RATE	1.0	12.1	1.4	0.0	0.1	5.8	
RINSE NO. 1	0.5	5.6	0.7	0.0	0.1	0.0	
PRODUCT (50%)	0.5	2046.7	4.0	51.1	77.7	50.4	
PRODUCT 2X	0.5	110.1	2.2	50.5	0.0	0.0	
PRODUCT (70%)	0.5	157.4	0.2	156.6	9.8	2.0	
PRODUCT 2X	0.5	150.1	0.0	0.0	0.0	0.0	
RINSE NO. 2	0.5	0.9	0.1	0.8	0.0	0.0	
2.5% CAUSTIC	0.5	1.1	63.6	0.0	0.0	320.6	
RINSE NO. 3	0.5	1.2	40.5	0.0	0.0	336.2	
TOTAL					77.7	100.0	

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Witnessed and Understood by Me

Date

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Date

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## ISOFLAVONE HIGH PURITY STEP-WISE ELUTION TESTS

THE PURPOSE OF THESE EXPERIMENTS IS TO  
DETERMINE IF RESINS (ADD 000, DCA-11 & OF-050) GIVE A HIGHER PURITY PRODUCT FROM PLANT STRIPPER PRODUCT DILUTED  
WITH DCA-11 & pH ADJUSTED UTILIZING A STEP-WISE ELUTION OF 20% DCA-11 FOLLOWED BY A 70% ELUTION WASHING

## EXPERIMENTAL DATA

EXPERIMENT NUMBER 8 DATE 11/23/01  
FEED PLANT STRIPPER PRODUCT (DILUTED 1:3 WITH DCA-11)  
COND'S pH ADJUSTED 8.3  
CONCENTRATIONS FEED RATE 12 ML/MIN  
COLUMN TEMP 65 DEG C  
RESIN ADD 000  
LOADING 13.620 g/L resin  
ISOFLAVONE CONC IN FEED 60110 PPM  
ISOFLAVONE CONC IN FEED 0.001 g/L  
BED VOLUME FED 20 BV

FEED	Volume (L)	ISOFLAVONE (mg/L)	D/D (g/L)	Yield %	Purity %	Yield %	D/D Account
RAFFINATE	2.0	6011	1.8	55.8	100.00	100.00	
RINSE NO 1	0.5	2101	0.7	17.3	0.00	0.00	
PRODUCT 2X (20%)	0.5	10691	2.2	62.2	0.00	0.00	
PRODUCT 2X (70%)	0.5	314	0.0	0.0	1.15	0.00	
PRODUCT 2X (70%)	0.5	171	0.0	0.0	0.00	0.00	
RINSE NO 2	0.5	71	0.0	0.0	0.16	0.00	
2.5% CAUSTIC	0.5	57	491	0.0	0.13	166.26	
RINSE NO 3	0.5	74	143	0.1	0.16	112.69	
TOTAL					68.55	572.11	

EXPERIMENT NUMBER 9 DATE 11/23/01  
FEED PLANT STRIPPER PRODUCT (DILUTED 1:3 WITH DCA-11)  
COND'S pH ADJUSTED 8.3  
CONCENTRATIONS FEED RATE 12 ML/MIN  
COLUMN TEMP 65 DEG C  
RESIN 000  
LOADING 13.620 g/L resin  
ISOFLAVONE CONC IN FEED 60110 PPM  
ISOFLAVONE CONC IN FEED 0.001 g/L  
BED VOLUME FED 20 BV

FEED	Volume (L)	ISOFLAVONE (mg/L)	D/D (g/L)	Yield %	Purity %	Yield %	D/D Account
RAFFINATE	2.0	6011	1.8	55.8	100.00	100.00	
RINSE NO 1	0.5	147	0.6	2.5	0.02	0.00	
PRODUCT 2X (20%)	0.5	21475	4.0	61.2	69.84	52.63	
PRODUCT 2X (20%)	0.5	12702	1.9	66.0	0.00	0.00	
PRODUCT 2X (70%)	0.5	176.6	0.4	44.7	6.66	0.00	
PRODUCT 2X (70%)	0.5	1132	0.2	56.6	0.00	0.00	
RINSE NO 2	0.5	78	0.1	7.9	0.17	0.78	
2.5% CAUSTIC	0.5	66	417	0.0	0.16	161.04	
RINSE NO 3	0.5	5	24	0.2	0.11	10.00	
TOTAL					80.60	220.78	

EXPERIMENT NUMBER 10 DATE 11/23/01  
FEED PLANT STRIPPER PRODUCT (DILUTED 1:3 WITH DCA-11)  
COND'S pH ADJUSTED 8.3  
CONCENTRATIONS FEED RATE 12 ML/MIN  
COLUMN TEMP 65 DEG C  
RESIN DCA-11  
LOADING 13.620 g/L resin  
ISOFLAVONE CONC IN FEED 60110 PPM  
ISOFLAVONE CONC IN FEED 0.001 g/L  
BED VOLUME FED 20 BV

FEED	Volume (L)	ISOFLAVONE (mg/L)	D/D (g/L)	Yield %	Purity %	Yield %	D/D Account
RAFFINATE	2.0	6011	1.8	55.8	100.00	100.00	
RINSE NO 1	0.5	7.6	0.6	1.3	0.14	0.00	
PRODUCT 2X (20%)	0.5	18678	3.1	64.1	72.06	40.79	
PRODUCT 2X (20%)	0.5	10241	1.7	60.2	0.00	0.00	
PRODUCT 2X (70%)	0.5	418.4	0.7	59.9	13.30	0.21	
PRODUCT 2X (70%)	0.5	220.8	0.2	76.9	0.00	0.00	
RINSE NO 2	0.5	10.7	0.1	18.7	0.43	0.70	
2.5% CAUSTIC	0.5	10.7	0.1	18.7	0.43	0.70	
RINSE NO 3	0.5	10.7	0.1	18.7	0.43	0.70	
TOTAL					80.31	610.79	

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EXPERIMENT NUMBER: 13

FEED: PLANT STRIPPER PRODUCT (DILUTED 1:3 WITH DI H2O)  
COND'S: pH "ADJUSTED" 9.3

CONDITIONS: FEED RATE:  
COLUMN TEMP.:  
RESIN: SP-207

12 MLS/MIN  
65 DEG C  
D/S

LOADING: 18.078 g/L resin  
ISOFLAVONE CONC. IN FEED: 903.90 PPM  
ISOFLAVONE CONC. IN FEED: 0.904 g/L  
BED VOLUMES FED: 20 B.V.

BED VOLUME: 100 ml

	Volume (L)	Isoflavone (mg/kg)	D/S (g/kg)	Vol/g	Purity %	Yield %	D/S Account.
FEED	2.0	903.9	21		43.0	100.00	100.00
RAFFINATE	2.0	11.3	0.7		1.6	1.25	33.33
RINSE NO. 1	0.3	13.8	0.4		3.5	0.23	2.86
COMPOSITE (35%)	0.5	3348.4	0.9		372.0	92.61	10.71
PRODUCT 2X (35%)		1738.7	2.7		84.4	0.00	0.00
COMPOSITE (70%)	0.5	147.7	0.6		24.6	4.69	7.14
PRODUCT 2X (70%)		71.3	1.0		7.1	0.00	0.00
RINSE NO. 2	0.3	8.1	0.1		5.1	0.08	0.71
25% CAUSTIC	0.3	5.3	64.7		0.0	0.08	258.57
RINSE NO. 3	0.3	4.2	35.6		0.0	0.07	254.28
TOTAL						98.42	587.62

EXPERIMENT NUMBER: 14

FEED: PLANT STRIPPER PRODUCT (DILUTED 1:3 WITH DI H2O)  
COND'S: pH "ADJUSTED" 9.3

CONDITIONS: FEED RATE:  
COLUMN TEMP.:  
RESIN: OPTIPORE

12 MLS/MIN  
65 DEG C

D/S

LOADING: 18.078 g/L resin  
ISOFLAVONE CONC. IN FEED: 903.90 PPM  
ISOFLAVONE CONC. IN FEED: 0.904 g/L  
BED VOLUMES FED: 20 B.V.

BED VOLUME: 100 ml

	Volume (L)	Isoflavone (mg/kg)	D/S (g/kg)	Vol/g	Purity %	Yield %	D/S Account.
FEED	2.0	903.9	21		43.0	100.00	100.00
RAFFINATE	2.0	3.6	0.5		0.4	0.40	42.86
RINSE NO. 1	0.3	3.7	0.4		0.9	0.06	2.86
COMPOSITE (35%)	0.5	3688.7	3.0		63.0	92.61	35.71
PRODUCT 2X (35%)		924.7	1.3		71.1	0.00	0.00
COMPOSITE (70%)	0.5	1166	1.2		97.2	32.25	14.29
PRODUCT 2X (70%)		635.6	1.3		48.9	0.00	0.00
RINSE NO. 2	0.3	52.1	0.8		6.5	0.86	5.71
25% CAUSTIC	0.3	7.7	32.4		0.0	0.15	27.86
RINSE NO. 3	0.3	12.2	86.3		0.0	0.20	816.43
TOTAL						98.42	587.62

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## ISOFLAVONE HIGH PURITY STEP-WISE ELUTION TESTS

THE PURPOSE OF THESE EXPERIMENTS IS TO:

DETERMINE IF RESINS, (AD9 600,DCA-11 & SP-850) GIVE A HIGHER PURITY PRODUCT FROM PLANT STRIPPER PRODUCT DILUTED WITH DI H<sub>2</sub>O & pH ADJUSTED UTILIZING A STEP-WISE ELUTION OF 35% E10H FOLLOWED BY A 70% ELUTION WASHING.

## EXPERIMENTAL DATA

EXPERIMENT NUMBER: 11

DATE: 10/24/01

FEED: PLANT STRIPPER PRODUCT (DILUTED 1:3 WITH DI H<sub>2</sub>O)

COND'S: pH "ADJUSTED" 9.3

CONDITIONS:

FEED RATE:

12 MLS/MIN

COLUMN TEMP.:

65 DEG C

RESIN: AD9 600

0.52 D/S

LOADING: 18.078 g/L resin

BED VOLUME: 100 ml

ISOFLAVONE CONC. IN FEED: 903.90 PPM

ISOFLAVONE CONC. IN FEED: 0.904 g/L

BED VOLUMES FED: 20 B.V.

	Volume (L)	Isoflavone (mg/kg)	D/S (g/kg)	Vol/g	Purity %	Yield %	D/S Account.
FEED	2.0	903.9	2.1		43.0	100.00	100.00
RAPPINATE	2.0	19.9	0.6		3.3	2.20	28.51
RINSE NO. 1	0.5	26.7	0.3		0.4	0.14	2.14
COMPOSITE (35%)	0.5	341.3	1.4		7.4	60.88	62.35
PRODUCT 2X (35%)		1590.6	2.5		63.6	0.00	0.00
COMPOSITE (70%)	0.5	14.4	0.0		0.0	0.40	0.00
PRODUCT 2X (70%)		10.7	0.1		0.0	0.00	0.00
RINSE NO. 2	0.5	6.3	0.0		0.0	0.10	0.00
2.5% CAUSTIC	0.5	8.6	0.1		0.0	0.14	325.71
RINSE NO. 3	0.5	3.7	0.0		0.0	0.06	121.43
TOTAL						100.00	550.24

EXPERIMENT NUMBER: 12

FEED: PLANT STRIPPER PRODUCT (DILUTED 1:3 WITH DI H<sub>2</sub>O)

COND'S: pH "ADJUSTED" 9.3

CONDITIONS:

FEED RATE:

12 MLS/MIN

COLUMN TEMP.:

65 DEG C

RESIN: SAFIZA

LOADING: 18.078 g/L resin

BED VOLUME: 100 ml

ISOFLAVONE CONC. IN FEED: 903.90 PPM

ISOFLAVONE CONC. IN FEED: 0.904 g/L

BED VOLUMES FED: 20 B.V.

	Volume (L)	Isoflavone (mg/kg)	D/S (g/kg)	Vol/g	Purity %	Yield %	D/S Account.
FEED	2.0	903.9	2.1		43.0	100.00	100.00
RAPPINATE	2.0	14.1	0.6		0.7	0.45	28.51
RINSE NO. 1	0.5	5.8	0.2		2.9	0.10	1.43
COMPOSITE (35%)	0.5	6.7	0.0		0.0	0.19	0.00
PRODUCT 2X (35%)		6.6	0.0		0.0	0.00	0.00
COMPOSITE (70%)	0.5	3.9	0.0		0.0	0.11	0.00
PRODUCT 2X (70%)		3.9	0.1		3.9	0.00	0.00
RINSE NO. 2	0.5	4.5	0.2		2.3	0.07	1.43
2.5% CAUSTIC	0.5	22.5	0.3		0.0	0.04	9.29
RINSE NO. 3	0.5	5.5	0.1		0.0	0.06	107.14
TOTAL						100.00	547.88

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## ISOFLAVONE HIGH PURITY STEP WISE ELUTION TESTS

THE PURPOSE OF THESE EXPERIMENTS IS TO:

DETERMINE IF RESINS, (ADS 600,DCA-11 &amp; SP-850) GIVE A HIGHER PURITY PRODUCT FROM PLANT STRIPPER PRODUCT DILUTED WITH DI H2O &amp; pH ADJUSTED UTILIZING A GRADIENT ELUTION OF 35% ETOH FOLLOWED BY A 70% ELUTION WASHING.

## EXPERIMENTAL DATA

EXPERIMENT NUMBER 15

DATE: 10/25/01

FEED: PLANT STRIPPER PRODUCT (DILUTED 1:3 WITH DI H2O)

COND'S: pH "ADJUSTED" 9.3

CONDITIONS:

FEED RATE:

12 MLS/MIN

COLUMN TEMP:

65 DEG C

RESIN: ADS 600

D/S

LOADING: 19.636 g/L resin

BED VOLUME: 100 mls

ISOFLAVONE CONC. IN FEED: 981.80 PPM

ISOFLAVONE CONC. IN FEED: 0.982 g/L

BED VOLUMES FED: 20 B.V.

	Volume (L)	ISOFLAVONE(mg/kg)	D/S(g/kg)	Vol/g	Purity %	Yield %	D/S Account.
FEED	2.0	981.8	2.0		0.0	100.00	0.01
RAFFINATE	2.0	23.4	0.0		0.0	2.38	0.01
RINSE NO.1	0.3	22.5	0.0		0.0	0.75	0.01
COMPOSITE	0.5	3057.0	4.5		71.1	71.84	0.06
PRODUCT 2X		1130.5	1.4		80.8	0.00	0.00
COMPOSITE	0.5	584.3	0.5		0.0	14.88	0.00
PRODUCT 2X		322.7	0.3		0.0	0.00	0.00
RINSE NO. 2	0.3	45.6	0.0		0.0	0.70	0.00
2.5% CAUSTIC	0.3	83.1	43.7		0.1	0.51	0.00
RINSE NO. 3	0.3	14.0	38.9		0.0	0.21	0.00
TOTAL						96.87	0.00

EXPERIMENT NUMBER: 16

FEED: PLANT STRIPPER PRODUCT (DILUTED 1:3 WITH DI H2O)

COND'S: pH "ADJUSTED" 9.3

CONDITIONS:

FEED RATE:

12 MLS/MIN

COLUMN TEMP:

65 DEG.C.

RESIN: HP20

LOADING: 19.636 g/L resin

BED VOLUME: 100 mls

ISOFLAVONE CONC. IN FEED: 981.80 PPM

ISOFLAVONE CONC. IN FEED: 0.982 g/L

BED VOLUMES FED: 20 B.V.

	Volume (L)	ISOFLAVONE(mg/kg)	D/S(g/kg)	Vol/g	Purity %	Yield %	D/S Account.
FEED	2.0	981.8	2.0		0.0	100.00	0.00
RAFFINATE	2.0	30.8	10.7		4.4	3.10	0.00
RINSE NO.1	0.3	88.1	0.4		16.5	1.01	0.00
PRODUCT(35%)	0.5	318.1	2.7		115.4	79.35	0.00
PRODUCT 2X		118.1	0.0		0.0	0.00	0.00
PRODUCT(70%)	0.5	389.6	0.7		55.7	9.92	0.00
PRODUCT 2X		198.5	0.0		0.0	0.00	0.00
RINSE NO. 2	0.3	12.4	0.4		3.1	0.19	0.00
2.5% CAUSTIC	0.3	16.3	4.6		0.0	0.10	0.00
RINSE NO. 3	0.3	0.0	28.0		0.0	0.01	0.00
TOTAL						93.71	0.00

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EXPERIMENT NUMBER:

17

FEED: PLANT STRIPPER PRODUCT (DILUTED 1:3 WITH DI H<sub>2</sub>O)

COND'S: pH "ADJUSTED"

9.3

CONDITIONS:

FEED RATE:

12 mL3/MIN

COLUMN TEMP.:

48.5 DEG C

RESIN: XAD7HP

D/S

LOADING: 19.636 g/L resin

BED VOLUME:

100 mls

ISOFLAVONE CONC. IN FEED: 981.80 PPM

ISOFLAVONE CONC. IN FEED: 0.982 g/L

BED VOLUMES FED: 20 B.V.

	Volume (L)	Isoflavone (mg/kg)	D/S (g/kg)	Vol./g	Purity %	Yield %	D/S Account.
FEED	2.0	981.8	2.0		49.1	100.00	0.00
RAFFINATE	2.0	37.3	1.1		3.4	3.80	0.00
RINSE NO. 1	0.3	84.3	1.0		0.4	0.98	0.00
PRODUCT (35%)	0.5	2866.4	5.0		57.3	72.99	0.00
PRODUCT 2X		1022	1.8		56.8	0.00	0.00
PRODUCT (70%)	0.5	701.7	1.0		70.2	17.87	0.00
PRODUCT 2X		384.4	0.8		64	0.00	0.00
RINSE NO. 2	0.3	94.7	0.4		23.7	1.46	0.00
2.5% CAUSTIC	0.3	183.4	61.7		0.3	2.80	0.00
RINSE NO. 3	0.3	0	0.0		0.0	0.00	0.00
TOTAL						98.89	0.00

EXPERIMENT NUMBER:

18

FEED: PLANT STRIPPER PRODUCT (DILUTED 1:3 WITH DI H<sub>2</sub>O)

COND'S: pH "ADJUSTED"

9.3

CONDITIONS:

FEED RATE:

12 mL3/MIN

COLUMN TEMP.:

65 DEG C

RESIN: XAD4/1090

D/S

LOADING: 19.636 g/L resin

BED VOLUME:

100 mls

ISOFLAVONE CONC. IN FEED: 981.80 PPM

ISOFLAVONE CONC. IN FEED: 0.982 g/L

BED VOLUMES FED: 20 B.V.

	Volume (L)	Isoflavone (mg/kg)	D/S (g/kg)	Vol./g	Purity %	Yield %	D/S Account.
FEED	2.0	981.8	2.0		49.1	100.00	100.00
RAFFINATE	2.0	20.8	33.4		0.1	2.12	1870.00
RINSE NO. 1	0.3	15.9	0.7		2.1	0.25	15.25
PRODUCT (35%)	0.5	3318	8.3		87.5	84.44	17.50
PRODUCT 2X		866.8	1.7		52.8	0.00	0.00
PRODUCT (70%)	0.5	508.5	0.8		63.6	12.95	10.00
PRODUCT 2X		313.4	0.1		313.4	0.00	0.00
RINSE NO. 2	0.3	34.1	0.3		11.4	0.62	2.25
2.5% CAUSTIC	0.3	26.1	61.2		0.0	0.40	271.50
RINSE NO. 3	0.3	11.4	42.4		0.0	0.17	318.00
TOTAL						100.85	2224.50

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## ISOFLAVONE HIGH PURITY STEP-WISE ELUTION TESTS

THE PURPOSE OF THESE EXPERIMENTS IS TO:

DETERMINE IF RESINS, (ADS 600,DCA-11 &amp; SF-850) GIVE A HIGHER PURITY PRODUCT FROM PLANT STRIPPER PRODUCT DILUTED WITH DI H2O &amp; pH ADJUSTED UTILIZING A GRADIENT ELUTION OF 35% ETOH FOLLOWED BY A 70% ELUTION WASHING.

## EXPERIMENTAL DATA

EXPERIMENT NUMBER:

19

DATE: 10/26/01

FEED: PLANT STRIPPER PRODUCT (DILUTED 1:3 WITH DI H2O)

COND'S: pH "ADJUSTED"

9.3

CONDITIONS:

FEED RATE:

12 MLS/MIN

COLUMN TEMP:

65 DEG C

RESIN: ADS 600

D/S

LOADING: 18,154 g/L resin

BED VOLUME: 100 ml

ISOFLAVONE CONC. IN FEED: 1067.90 PPM

ISOFLAVONE CONC. IN FEED: 1.068 g/L

BED VOLUMES FED: 17 B.V.

	Volume (L)	ISOFLAVONE(mg/kg)	D/S(g/kg)	Vol/g	Purity %	Yield %	D/S Account.
FEED	1.7	1067.9	1.6		66.7	100.00	100.00
RAFFINATE	1.7	23.7	0.6		4.0	2.22	37.50
RINSE NO. 1	0.3	21.9	0.5		2.3	0.36	1.31
COMPOSITE	0.5	2822.8	3.5		80.3	77.91	64.34
PRODUCT 2X		1430.2	1.7		84.1	0.00	0.00
COMPOSITE	0.5	133.8	0.2		60.9	3.69	3.69
PRODUCT 2X		287.4	0.2		143.7	0.00	0.00
RINSE NO. 2	0.3	39.4	0.2		19.7	0.65	2.21
2.5% CAUSTIC	0.3	31.3	51.6		0.1	0.52	270.59
RINSE NO. 3	0.3	4.4	26.9		0.2	0.07	296.69
TOTAL						25.42	1678.31

EXPERIMENT NUMBER:

20

FEED: PLANT STRIPPER PRODUCT (DILUTED 1:3 WITH DI H2O)

COND'S: pH "ADJUSTED"

9.3

CONDITIONS:

FEED RATE:

12 MLS/MIN

COLUMN TEMP:

65-DEG C

RESIN: SF700

D/S

LOADING: 21358 g/L resin

BED VOLUME: 100 ml

ISOFLAVONE CONC. IN FEED: 1067.90 PPM

ISOFLAVONE CONC. IN FEED: 1.068 g/L

BED VOLUMES FED: 20 B.V.

	Volume (L)	ISOFLAVONE(mg/kg)	D/S(g/kg)	Vol/g	Purity %	Yield %	D/S Account.
FEED	2.0	1067.9	1.6		66.7	100.00	100.00
RAFFINATE	2.0	12.3	0.6		2.1	1.5	37.50
RINSE NO. 1	0.3	17.3	0.4		4.3	0.24	3.75
PRODUCT(35%)	0.5	3892.2	5.8		67.1	91.12	90.63
PRODUCT 2X		1979.4	2.8		70.7	0.00	0.00
PRODUCT(70%)	0.5	460.4	0.5		92.1	10.78	7.81
PRODUCT 2X		222.8	0.2		111.4	0.00	0.00
RINSE NO. 2	0.3	39.3	0.1		39.3	0.55	0.94
2.5% CAUSTIC	0.3	17.4	39.9		0.1	0.01	14.69
RINSE NO. 3	0.3	4.3	27.6		0.8	0.06	258.75
TOTAL						104.01	514.06

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# ISOFLAVONE HIGH PURITY LOADING VS. PERFORMANCE TESTS

THE PURPOSE OF THESE EXPERIMENTS IS TO:

DETERMINE IF A VARIANCE IN THE LOADING BED VOLUME OF AD5600 GIVES A HIGHER PURITY PRODUCT

## EXPERIMENTAL DATA

EXPERIMENT NUMBER: 31

DATE: 10/3/01

FEED: PLANT STRIPPER PRODUCT (DILUTED 1:3 WITH DI H<sub>2</sub>O)

COND'S: pH "ADJUSTED" 9.3

CONDITIONS:

FEED RATE:

12 MLS/MIN

COLUMN NO.: 1

COLUMN TEMP:

65 DEG C

RESIN:

AD5600

LOADING: 7.9 g/L resin

BED VOLUME: 100 ml

ISOFLAVONE CONC. IN FEED: 785.1 PPM

ISOFLAVONE CONC. IN FEED: 0.79 g/L

BED VOLUMES FED: 10 B.V.

	Volume (L)	Isoflavone(mg/kg)	D/S(g/kg)	Vol/g	Purity %	Yield %	D/S Account.
COLUMN FEED	1.0	785.1	1.6		49.1	100.0	100.0
RAFFINATE	1	16.4	0.9		1.8	2.1	56.5
RINSE NO. 1	0.3	17.0	0.0		0.0	0.8	0.0
PRODUCT (35%)	0.5	1804.9	1.9		95.0	114.9	59.4
PRODUCT 2X (35%)	0.5	903.5	0.9		100.9	0.0	0.0
PRODUCT (70%)	0.5	6.7	0.0		0.0	0.4	0.0
PRODUCT 2X (70%)		4.7	0.0		0.0	0.0	0.0
RINSE NO. 2	0.3	0.7	0.0		0.0	0.0	0.0
NaOH	0.3	2.4	63.9		0.0	0.1	704.4
RINSE NO. 3	0.3	1.6	5.3		0.0	0.1	99.4
TOTAL					100.0	919.4	

## EXPERIMENTAL DATA

EXPERIMENT NUMBER: 32

DATE: 10/3/01

FEED: PLANT STRIPPER PRODUCT (DILUTED 1:3 WITH DI H<sub>2</sub>O)

COND'S: pH "ADJUSTED" 9.3

CONDITIONS:

FEED RATE:

12 MLS/MIN

COLUMN NO.: 1

COLUMN TEMP:

65 DEG C

RESIN:

AD5600

LOADING: 15.7 g/L resin

BED VOLUME: 100 ml

ISOFLAVONE CONC. IN FEED: 785.1 PPM

ISOFLAVONE CONC. IN FEED: 0.79 g/L

BED VOLUMES FED: 20 B.V.

	Volume (L)	Isoflavone(mg/kg)	D/S(g/kg)	Vol/g	Purity %	Yield %	D/S Account.
COLUMN FEED	2.0	785.1	1.6		49.1	100.0	100.0
RAFFINATE	2	24.9	0.9		5.2	3.0	31.3
RINSE NO. 1	0.3	36.1	0.2		19.0	0.7	1.9
PRODUCT (35%)	0.5	3159.6	3.7		85.4	100.6	57.8
PRODUCT 2X (35%)		1563.6	1.6		97.7	0.0	0.0
PRODUCT (70%)	0.5	180.5	0.1		180.5	5.7	1.6
PRODUCT 2X (70%)		104.6	0.2		52.3	0.0	0.0
RINSE NO. 2	0.3	7.1	0.1		7.1	0.1	0.9
NaOH	0.3	3.0	11.7		10.0	0.1	31.6
RINSE NO. 3	0.3	15.4	1.6		0.1	0.3	103.2
TOTAL						110.7	408.8

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